IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Bror Morein et al.
Appln. No.	: 10/550,026
Filed	: June 11, 2007
Title	: COMPOSITION COMPRISING ISCOM PARTICLES AND LIVE MICRO-ORGANISMS
Conf. No.	: 6185
TC/A.U.	: 1648
Examiner	: Zachariah Lucas
Customer no.	: 00116
Docket No.:	: ALBI-41848

DECLARATION UNDER 37 CFR 1.132

Sir:

This Declaration under 37 CFR 1.132 is filed in response to the outstanding Office action of December 17, 2008.

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DECLARATION OF BROR MOREIN

Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Bror Morein, having knowledge of the facts set forth herein, declares as follows:

1. Exhibits A, B, C, and D are attached and are made part of this declaration.

2. I presently reside at Ollonstigen 3, Uppsala, Sweden.

3. I am a co-inventor of the subject matter claimed in the above-captioned patent application.

4. My qualifications include the following. I earned a Ph.D. in Virology at the Royal Veterinary College, Stockholm in 1973. I achieved the position of Professor of Virology of the Swedish University of Agricultural Sciences in 1981. I became Senior Research Director of Isconova AB in 2002. My current titles include Visiting Professor of the Department of Virology, Uppsala University, and Chairman, Scientific Advisory Board ISCONOVA AB, both since 2004. Additional details of my qualifications are included in Exhibit D.

5. I have authored or co-authored more than 200

scientific research publications in my field, including numerous publications regarding iscom research and analysis. Selected and recent articles are listed in Exhibit D.

6. I am the inventor or co-inventor of approximately 20 patents and patent applications in the fields of vaccinology, monoclonal antibodies, and drug delivery. Selected recent patents and patent applications, including ones directed to iscom technology, are listed in Exhibits C and D.

7. Adjuvants are used in killed vaccines to enhance immunogenicity of the vaccine antigens. Killed vaccines, particularly subunit vaccines, are far less immunogenic and require the addition of an adjuvant to reach acceptable immunity compared to, for example, live attenuated vaccines that replicate in a controlled manner to give a subclinical infection stimulating long-lived immunity.

8. The iscom technology is an adjuvant technology developed and designed for subunit antigen preparations. The iscom technology was developed during the 1980's as a potent mean of conferring adjuvant activity to selected purified antigens obtained from extraction of microorganisms or as recombinant-DNA products.

9. It was previously known to combine an iscom matrix particle with a subunit antigen to yield an iscom particle for use, e.g., as a vaccine. In contrast, it was not previously known to combine an iscom matrix particle or an iscom particle (hereinafter also termed "iscom matrix/iscom particle") with a live microorganism to form a single composition for use, e.g., as a vaccine.

10. Of note, the technology published and used for making iscom particles from whole microorganisms from before 2004 and even to date, including the technology described in the

references to Wechter (U.S. Pat. No. 6,177,081) and Van Woensel (U.S. Pat. No. 5,925,359) as cited by the Examiner, is based on extraction of antigens from the whole microorganisms followed by incorporation of the antigens into iscom matrix particles to yield iscom particles, not based on incorporation of whole organisms per se into iscom particles. More specifically, solubilization procedures are used for incorporation of antigens into iscom matrix particles. The solubilization procedures include use of agents, typically detergents, to disintegrate or to selectively extract surface antigens out of the microbial structure for subsequent incorporation into iscom matrix particles to yield iscom particles. The solubilization procedures used for extraction will damage and disintegrate whole microorganisms, thus killing any live microorganisms and preventing the microorganisms from subsequently replicating. Since microorganisms subject to such solubilization treatments are no longer living, such solubilization treatments do not permit incorporation of live microorganisms into iscom matrix particles and do not result in compositions including an iscom matrix particle or an iscom particle and a whole microorganism, let alone a live microorganism. Moreover, iscom matrix particles and iscom particles are only approximately 40 nm in size. The size of iscom matrix/iscom particles does not permit the incorporation of microbial units larger than protein molecules into such particles, and thus does not permit incorporation of whole microorganisms, whether live or not. For example, viruses are generally in the range of 20 to 300 nm, bacteria are generally in the range of several hundred nanometers to several micrometers, and fungi and parasites are generally even larger in size.

11. Regarding the Wechter reference in particular, although the Wechter reference uses the words "live attenuated

viruses," col. 9, lines 29-30, the skilled person reading the full paragraph of the Wechter reference from lines 28 to 44 will recognize that the Wechter reference itself indicates that there are no live viruses whatsoever left in the composition resulting from the incorporation and ultimately used for immunization. Specifically, the Wechter reference states the following:

> The protective effects of most vaccines are due to induced levels of circulating antibodies. Live attenuated viruses can also be incorporated into immunostimulating complexes (ISCOM) for use as a vaccine using methods well known in the art. Activating Virus immunostimulating complexes (ISCOM)-vaccine containing viral capsid proteins to raise high neutralization antibody titres after two booster doses in Balb/c CUM and *NJ* inbred mouse strains. Fohlman, et al., supra. The presentation of viral coat protein antigens in ISCOM particles has three main advantages: no replicating viral nucleic acid is introduced into the host, high levels of neutralizing antibodies are achieved, and a cellular immunity is evoked, including cytotoxic T-cells induced under restriction of MHC class II. The methodology for making ISCOM vaccines is well known in the art. (B. Morein, et al., Nature, 308:457-60, 1984).

Wechter, col. 9, lines 28-44. The quoted passage indicates that one advantage of incorporating live attenuated viruses into iscom matrix particles to yield iscom particles is that, upon subsequent administration of such iscom particles, no viral nucleic acid is introduced into the host. This is an advantage that could not be realized if the Wechter reference were disclosing a vaccine that included both a live attenuated virus and iscom particles. The quoted passage also indicates that live attenuated viruses can be incorporated into iscom matrix particles by methods well known in the art, and that the methodology for making iscom vaccines is well known in the art. As indicated above, no method for integration of a live microorganism per se into an iscom matrix particle was known, let alone well known, in the art. Moreover, no methodology

for making iscom vaccines that include iscom matrix/iscom particles and a live attenuated virus was known.

12. Regarding the Van Woensel reference in particular, although Van Woensel indicates that "[i]ncorporation of the antigens in Iscoms is also a possible way of adjuvation," col. 5, lines 18-19, a person of ordinary skill in the art reading the statement would realize that Van Woensel does not disclose incorporation of a live attenuated virus, or any whole microorganism, into an iscom matrix/iscom particle. As indicated above, the technology used for production of iscom particles from whole live/attenuated or killed microorganisms is well documented in the literature and involves use of a solubilization agent that damages and disintegrates the microorganisms, causing loss of the ability of the microorganisms to infect or replicate. The result is that antigens of the microorganisms, not live microorganisms or whole microorganisms, are incorporated to yield iscom particles.

13. A person of ordinary skill in the art at the time the invention was made would not have been motivated to combine an iscom matrix/iscom particle with a live microorganism to form a single composition, for at least the following reasons. First, most other commonly used adjuvants were known to decrease the capacity of live microorganisms to replicate. Second, iscom matrix/iscom particles were known to preferably be made with saponins, and saponins were known to have a particularly striking anti-microbial nature. A person of ordinary skill in the art thus would have expected that adjuvants in general, and iscom matrix/iscom particles in particular, would have had a negative effect on replication of the live microorganism component of such a composition.

14. More specifically with regard to saponins, saponins

are surface active substances with a strong binding affinity to cholesterol. Saponins are therefore lytic and cause damage or disruption of cell or microbial membranes. Of note, even if the lytic effects of saponins can have a direct damaging effect on a whole range of microorganisms, it cannot explain many of the Molluscicidal, Antifungal, Virucidal, Antimicrobial and Antiparasitic effects reported in the literature. This is shown for example in Exhibit A, which corresponds to a research publication, Francis, G. et al., The Biological Action of Saponins in Animal Systems: A Review, British Journal of Nutrition (2002), 88, 587-605. See particularly pages 596 and 598. This is also shown, for example, in Exhibit B, which corresponds to another research publication, Sparg, S.G. et al., Biological Activities and Distribution of Plant Saponins, Journal of Ethnopharmacology (2004), 94, 219-243. See particularly pages 221-223.

15. Saponins were known to have membrane-permeabilizing activity, indicating a negative effect on live organisms, as shown for example in Exhibit A, page 598. Saponins were also known to be useful for their insecticidal, antibiotic, fungicidal and pharmacological properties , due to their toxicity to various organisms, as shown for example in Exhibit B, page 235. These references and others taught a person of ordinary skill in the art to expect that saponins would have a harmful and even killing effect on microorganisms. Thus, a person of ordinary skill in the art would not have been motivated to use saponins in any form together with live microorganisms.

16. Of note, although Quillaja saponins were known to have adjuvant activities, a person of ordinary skill in the art would have expected the negative effects of saponins on live microorganisms to have outweighed the positive effects of saponins as adjuvants and thus would not have been motivated

to combine saponins with live microorganisms in a single composition. For example, it was known that saponins increase the immune response to antigens when added to preparations of the antigens. It was also known that saponins increase immune cell proliferation in vitro. It was further known that mice fed with Quillaja saponins exhibited increased cell proliferation in spleen and mesenteric lymph nodes and exhibited increased and prolonged natural killer cell activity, suggesting a direct action on T helper cells of the mucosal immune system to induce secretion of soluble mediators. It was also known that oral feeding of saponins induced a non-specific resistance against live challenge infection with rabies virus. Nonetheless, as saponins were known to have negative effects against live microorganisms, saponins would have been expected to have particularly negative effects on live attenuated microorganisms, such as live attenuated viruses. Therefore, a person of ordinary skill in the art would not have been motivated to consider using any form of saponins together with live attenuated microorganisms. The prospects of a virus of a live attenuated vaccine to replicate and stimulate immune responses in the presence of a saponin based adjuvant did not look promising.

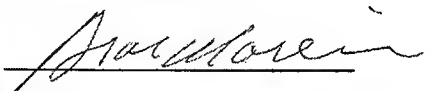
17. Of further note, I am a co-inventor of U.S. Pat. No. 5,679,354. For the reasons indicated above with regard to the negative effects of saponins on live microorganisms, when the inventors of the '354 patent, including myself, indicated in the '354 patent that iscom matrix could be used as an adjuvant with whole organisms, we did not intend that Quillaja saponin and/or iscom matrix/iscom particles would be used with live whole microorganisms.

18. Surprisingly and unexpectedly, the present inventors have discovered that iscom matrix/iscom particles can be used as an adjuvant with a live microorganism. The present

invention was the result of an unexpected finding while exploring the prospects of including a killed vaccine component requiring an adjuvant in a vaccine regimen based on live attenuated vaccines. The inventors had hoped that the adjuvant, iscom matrix particles, as present in a vaccine, would not significantly negatively affect live attenuated components if administered simultaneously but not necessarily together with the live components. Surprisingly and unexpectedly, as indicated in the present application, the inventors found that the iscom/iscom matrix adjuvant was not only harmless to the live components, but that it also enhanced the immune response against the live components.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001, and that such willful false statements may jeopardize the validity of the present application or any patent issued thereon.

Inventor Name: Bror Morein

Signature: 

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Date: May 18, 2009